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(54) Title: LIBRAIRIES OF BACKBONE-CYCLIZED PEPTIDOMIMETICS

(57) Abstract

Libraries of novel backbone-cyclized peptide analogs are formed by means of bridging groups attached via the alpha nitrogens of amino acid derivatives to provide novel non-peptidic linkages. Novel building units used in the synthesis of these backbone-cyclized peptide analogs are Nα (ω-functionalized) amino acids constructed to include a spacer and a terminal functional group. One or more of these Nα (ω-functionalized) amino acids are incorporated into a library of peptide sequences, preferably during solid phase peptide synthesis. The reactive terminal functional groups are protected by specific protecting groups that can be selectively removed to effect either backbone-to-backbone or backbone-to-side chain cyclizations. The invention is exemplified by libraries of backbone-cyclized bradykinin analogs, somatostatin analogs, BPI analogs and Substance P analogs having biological activity. Further embodiments of the invention are Interleukin-6 receptor derived peptides having ring structures involving backbone cyclization.

# LIBRARIES OF BACKBONE-CYCLIZED PEPTIDOMIMETICS

## Field of the Invention

This invention relates to libraries of conformationally constrained backbone cyclized peptidomimetics, to methods for the production of such libraries and to methods of using them to screen for biologically active compounds. Within the scope of this invention are certain novel conformationally constrained peptidomimetic molecules which are disclosed and claimed herein.

## Background of the Invention

### Peptide libraries

Classically, the pharmaceutical industry has screened a wide variety of compounds derived from natural sources to yield potential drug candidates or lead compounds for the development of new drugs. These laborious screening efforts have relied on the random testing of a vast number of chemical entities. In recent years, various strategies have been adopted for the generation of libraries of compounds that are subsequently screened as a novel, rational approach to drug discovery and development.

It has become apparent that a variety of methodologies can be applied to the problem of generating a diverse group of candidate compounds, based on the known principles of peptide chemistry and/or molecular biology. Peptides are a convenient class of molecules for the generation of combinatorial libraries, since they are composed of a finite set of amino acid building units, which can be efficiently assembled either by chemical synthesis or transcription/translation of DNA. Combinatorial libraries are discussed by Gallop et al., J. Med. Chem., 37, 1233-1251

(1994); Gordon et al., J. Med. Chem., 37, 1385-1401 (1994); Pinilla et al., Biopolymers (Peptide Science), 37, 221-240, (1995); and Lebl et al., Biopolymers (Peptide Science), 37, 177-198 (1995). The set of amino acid building units can include only the naturally encoded amino acids, when the libraries are encoded by oligonucleotides on a plasmid, phage, or any other vector. This set can be expanded to include both D and L amino acids and/or non-natural amino acids in synthetic libraries.

Linear peptides suffer from several serious drawbacks as potential drugs, inasmuch as they are notoriously unstable in vivo, often lack high affinity of binding to their receptor, frequently lack selectivity to one kind of receptor, and generally have poor oral bioavailability. In efforts to overcome such problems, it is also possible to utilize the methodologies developed in connection with synthetic peptide libraries to generate collections of cyclic peptides, novel biopolymers and even novel branched oligomeric compounds, reviewed by Zuckermann, Current Opinion in Structural Biology, 3, 580-584 (1993).

One of the most significant synthetic technologies that facilitate the generation and screening of diverse chemical libraries is the resin-splitting method, which is a polymer supported multiple synthesis procedure that allows a high degree of control over the composition of a peptide mixture. Mixtures are generated by dividing a solid support into individual portions, and coupling a different amino acid to each portion, and then recombining the portions. These steps may be performed in an iterative fashion to provide the required degree of diversity.

Totally random libraries generated by these types of methods are disclosed in WO92/00091 and WO92/09300. Each individual bead will contain a unique peptide sequence, which can be probed for activity with a soluble receptor or antibody. Positive beads can be isolated and sequenced using Edman sequencing chemistry. WO92/00091 further discloses methods to provide selectively cleavable linkers between

peptide and resin, such that part of the peptide can be liberated from the resin and assayed for activity in soluble form, while another part can be sequenced. In addition, it is also possible to generate random libraries in which each bead carries more than one peptide, by coupling of mixtures of amino acids to the beads, as disclosed by Hornik et al., Reactive Polymers, 22, 213-220 (1994).

Another methodology is disclosed by Geysen et al., J. Immunol. Meth., 102, 259-274 (1987), which involves the synthesis of peptides on derivatized polystyrene pins which are arranged in such a fashion that they correspond to the arrangement of wells in a 96-well microtiter plate. Individual chemical reactions can be performed in each well, thereby yielding individual peptides on each pin. The pins are typically probed using an enzyme linked immunoassay (ELISA) or radioimmunoassay (RIA), carried out in the microtiter wells, or the peptides may be released from the pins and tested in solution. The mimotope approach of Geysen et al. generates diverse peptides that are probed for activity in situ. The best dipeptide sequence is selected for elongation to diverse tripeptides, the best tripeptide is selected for elongation to a tetrapeptide and so on.

Ideally, chemistries that are amenable to combinatorial library synthesis would have the following characteristics: be polymer-supported to facilitate the resin splitting technique; be assembled in high yield with automatable chemistry; and allow the incorporation of a wide variety of chemical functionalities.

Cyclic peptides

Cyclic peptides are generally recognized as possessing enhanced bioavailability due to increased metabolic stability, as well as a relatively constrained conformation when compared to the same sequence in a linear form. The enhanced metabolic stability should allow diminished doses at longer intervals. The restricted conformation should improve the drug selectivity, thereby potentially preventing side-

effects. All of these properties are desirable in conjunction with the quest for new drug candidates.

The generation of libraries of cyclic peptides requires, in addition to any previously stated considerations, that the cyclization reaction be performed in a high yield and with a minimum of additional manipulations. Unfortunately, classical cyclization reactions are highly sequence dependent in terms of the expected yields, making the uniform cyclization of a peptide mixture unreliable.

Recent advances in the cyclization of peptides directly on the solid support have improved the synthetic procedure, and even allowed the automation of cyclization reactions based on known cyclization schemes. In the past, cyclizations were typically performed in solution under conditions of high dilution. Polymer-supported cyclizations can both avoid potential side reactions such as oligomerization and facilitate product purification. For example, on-resin cyclization methods have recently been used to prepare cyclopeptides with bridges formed of thioethers, disulfides, or lactams between two side chains, lactams between the amino terminus and a side chain, and lactams between the amino and carboxy termini (reviewed by Zuckermann, Current Opinion in Structural Biology, 3, 580-584 (1993).

The use of resin-bound cyclic peptides and free cyclic peptides in combinatorial libraries is disclosed in WO 92/00091. However, these cyclic peptides do not contain any conformationally constraining element, and in cases where cyclization is achieved, these peptides may still adopt a number of conformations and suffer many of the same shortcomings as linear peptides.

Cyclic semi-random peptide libraries, which are disclosed in WO 95/01800, are exclusively cyclic penta-peptide and hexa-peptide libraries containing one or more randomized amino acids and a conformationally constraining element in the form of an amino acid residue such as proline which fixes the beta turn angles of the adjacent amino acid residues. The advantages of such conformationally

constraining elements is stressed by the inventors of this approach. However, inclusion of such elements via incorporation of a particular amino acid residue into the peptide sequence may have detrimental effects on those residues required for receptor recognition or other biological activity. Furthermore, in WO 95/01800, the cyclization reaction is merely another coupling reaction in which the terminal amino group of the linear peptide is coupled to the terminal carboxy group of the peptide.

Backbone cyclized peptides

Backbone cyclized peptides are generally known, as discussed, for instance, in Gilon et al., Biopolymers, 31, 745-750 (1991); in EPO 564,739 A2; and in WO 95/33765 (PCT/IB95/00455). Such compounds have not been used for constructing libraries for screening purposes.

In addition, methods are known for combining amino acids into peptides. U.S. Patent 5,010,175 describes another method of incorporating random amino acids into a peptide. According to that method, a mixture of amino acids is incorporated by coupling a mixture in which the individual amino acids are present in varying proportions depending upon their relative rates of reaction in the coupling, e.g., the amount of amino acid is inversely proportional to its rate of coupling.

## Summary of the Invention

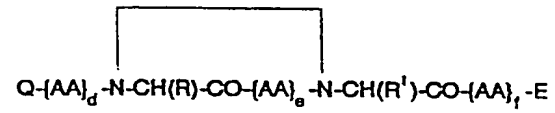
It is an object of this invention to provide backbone-cyclic peptide analog libraries that are suited for screening for bioactive molecules. This and other useful technology provided by the present invention is summarized below.

The present invention provides a library of chemical compounds that comprises a plurality of backbone-cyclized peptide analogs. Each compound in the library contains a peptide sequence having at least one building unit comprising an $N^{\alpha}$-derivative of an amino acid, and at least one backbone

nitrogen in each said peptide sequence is linked to a side chain of at least one other amino acid in the peptide sequence or to at least one other backbone nitrogen in the peptide sequence by a bridging group comprising a disulfide, amide, thioether, thioester, imine, ether, or alkene bridge to form a backbone-cyclized peptide analog. At least one of the building units is preferably located other than at the end of the peptide sequence, and more preferably, none of the building units is located at the end of the peptide sequence.

The newly generated libraries, disclosed according to the present invention, now enable screening for varying degrees of conformational constraint, in order to determine the optimal conformation of a peptide or peptide analog in performing its role as an agonist or antagonist. This is accomplished by generating a library of chemical compounds that comprises a plurality of backbone-cyclized peptide analogs, wherein the members of the library vary in: (i) the positions in the linear sequence of residues that are to be cyclized, (ii) the length of the bridge between these residues, (iii) direction of the bridge between these residues; and (iv) the bond type of the bridge between these residue. This novel type of library thereby enables the improvement of a known sequence of residues by screening for the optimal sequence of an active peptide analog.

According to one aspect of the invention, the library as described above comprises a plurality of backbone-cyclized peptide analogs, wherein at least one pair of backbone nitrogens in each peptide sequence is linked together to form a peptide analog having the general formula (I):



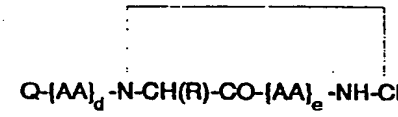

Formula (I)

wherein: d, e, and f each independently designates 0 or an integer from 1 to 10; each {AA} designates an amino acid residue or the residue of a plurality of amino acids linked together through peptide bonding, wherein each {AA} may be the same or different; Q represents H or an acyl group; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the carboxy terminal group CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH or CHO; each of R and R′ is independently hydrogen or an amino acid side-chain optionally bound with a specific protecting group; and the lines designate a bridging group of the formula:

(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein: M and W are independently selected from the group consisting of disulfide, amide, thioether, thioester, imine, ether, and alkene; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero-cycloalkylene and substituted cycloalkylene.

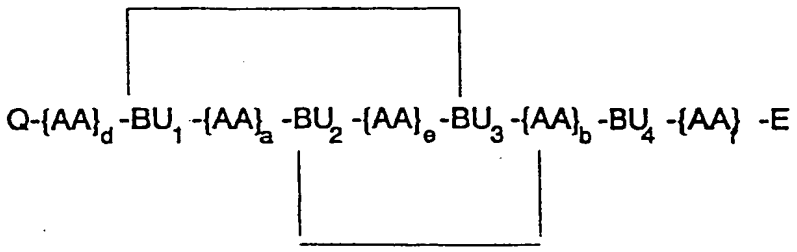
In another aspect of the invention, the library comprises a plurality of backbone-cyclized peptide analogs, wherein the backbone of each analog is cyclized to a side-chain of an amino acid to form a peptide analog of the general formula (II):




Formula (II)

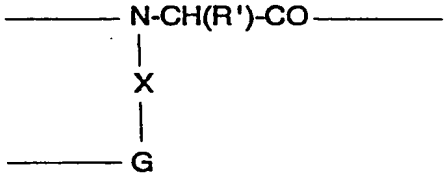
wherein the variables are as disclosed above.

A further library in accordance with the present invention comprises a plurality of backbone-cyclized bicyclic peptide analogs, each of which comprises a plurality of building units comprising an $N^{\alpha}$-derivative of an amino acid. Such bicyclic peptide analogs may have the formula (III):




Formula (III)

wherein each BU represents an $N^{\alpha}$-ω-functionalized derivative of amino acids of formula (IV):




Formula (IV)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R′ is an amino acid side chain, optionally bound with a specific protecting group; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, and alkyl halides; and the other variables are as disclosed above. The BU groups are incorporated into the peptide sequence and may subsequently be selectively cyclized via the functional group G with one of the side chains of the amino acids in said peptide sequence or with another ω-functionalized amino acid derivative.

It is preferred that libraries in accordance with the present invention, such as those described above, have at least four members. In certain preferred embodiments, at least some of the analogs are bradykinin analogs, Substance P analogs, BPI analogs, somatostatin analogs, or interleukin-6

inhibitory peptide analogs. In another preferred embodiment of the present invention, the library as described above comprises two or more sublibraries, each containing a plurality of related peptide analogs.

The present invention provides methods for screening for bioactive conformationally constrained peptide analogs. A method of screening for active conformers comprises: generating a library of chemical compounds that comprises a plurality of backbone-cyclized peptide analogs, wherein the members of the library vary in: (i) the positions in the linear sequence of residues that are to be cyclized, (ii) the length of the bridge between these residues, (iii) direction of the bridge between these residues, and (iv) the bond type of the bridge between these residues; screening the library for bioactivity; and identifying the active member or members of the library.

The present invention also provides methods for the preparation of libraries of chemical compounds as described above. The methods comprise the steps of: providing peptide sequences having a plurality of building units containing amino acids and linked nitrogen atoms and incorporating into each peptide sequence at least one $N^{\alpha}$-$\omega$-functionalized derivative of an amino acid of formula (IV) by selectively cyclizing a functional group G with another $\omega$-functionalized amino acid derivative or with one of the side chains of the amino acids in said peptide sequence to form backbone-cyclized peptide analogs.

Preferred embodiments for G in formula (IV) include amine, thiol, and carboxyl groups. Preferred embodiments for R and R′ in formulas (I)-(III) include CH3-, (CH3)2CH-, (CH3)2CHCH2-, CH3CH2CH(CH3)-, CH3S(CH2)2-, HOCH2-, CH3CH(OH)-, HSCH2-, NH2C(=O)CH2-, NH2C(=O)(CH2)2-, NH2(CH2)3-, HOC(=O)CH2-, HOC(=O)(CH2)2-, NH2(CH2)4-, C(NH2)2 NH(CH2)3-, HO-phenyl-CH2-, benzyl, methylindole, and methylimidazole.

A particularly useful embodiment of the present invention involves providing the peptide sequences as

described above covalently coupled to insoluble polymeric supports.

The present invention also provides a method of screening the compounds contained in the libraries for biochemical and biological activity. This method comprises forming a library of cyclized peptide analogs as described hereinabove and screening for those peptides that exhibit a similar activity to the natural peptide, or, alternatively, inhibit the activity of the natural peptide. For example, and not by way of limitation, some peptide analogs may act as agonists of the corresponding natural peptide's receptor, whereas other peptide analogs may act as antagonists of the corresponding natural peptide's receptor. These methods are exemplified herein for bradykinin analogs, Substance P analogs, BPI analogs, somatostatin analogs, and interleukin-6 inhibitory peptide analogs.

## Description of the Preferred Embodiments

In order to fully describe the present invention, the following definitions will be used.

A "library" of backbone cyclized peptide analogs indicates a collection of peptide analogs wherein at least one conformational constraint consisting of a bridge linking novel building units via modified side chains attached to the nitrogens of the amide bonds is present. Typically the amino acids in other positions of the peptide will be "variable" or "constant". Each library is characterized by its building units, its constant amino acid residues and its variable amino acid residues. Each library may be composed of "sub-libraries" which are synthesized in parallel, using a divergent or convergent synthetic scheme.

A "variable" position or amino acid residue may have more than one amino acid in the specified position of the peptide. Typically, in a set of sub-libraries, each sub-library differs from the other in the identity of at least one of its defined amino acid(s) (e.g., the defined amino acid(s) will be constant throughout a single sub-library, yet

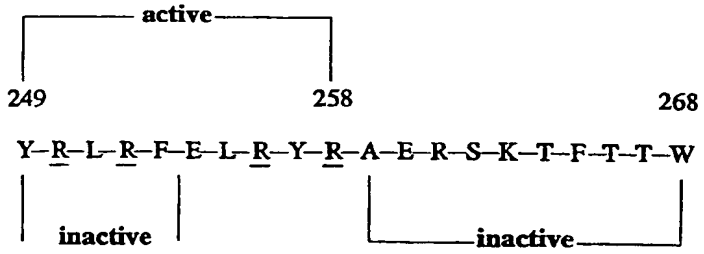
INTERLEUKIN-6 RECEPTOR PEPTIDE LIBRARIES:

Interleukin-6 (IL-6), also known as interferon-beta-2, is a pleiotropic cytokine, which acts as a growth and differentiation factor for a number of cell types. Overproduction of IL-6 has been implicated in the pathogenesis of multiple myeloma and in post-menopausal osteoporosis. Inhibition of the action of IL-6 should be of clinical benefit in the treatment of multiple myelomas, a malignancy in which the growth stimulatory effect of IL-6 contributes to tumor growth.

IL-6 is believed to interact sequentially with two transmembrane receptors, the low affinity IL-6 receptor (IL-6R alpha, also denoted gp80) and the signal transducer gp130, via distinct binding sites. The gp130 protein is also involved in signal transduction of a number of other growth factors or hormones (reviewed by Hirano et al., Stem Cells 12, 262-277, 1994).

It has been disclosed by Savino et al. (EMBO J. 13, 5863-5870, 1994; EMBO J. 13, 1357-1367, 1994), that site directed mutagenesis of IL-6 residues that are presumed to interact with the gp130 subunit, can yield antagonists that maintain unimpaired affinity to IL-6R alpha but no bioactivity due to inability to bind to gp130. Mutation of residues A229 and N231 were shown to prevent IL-6 signaling.

It has been further disclosed that inhibitory peptides may be designed to prevent interaction of IL-6 and its receptor. Grube and Cochrane (J. Biol. Chem. 269, 20791-20797, 1994) disclose a deca-peptide spanning residues 249 through 258 of the IL-6R molecule which is active in preventing the bioactivity of IL-6. This peptide, is more active then the 16- amino acids sequence Y249-T264. According to this disclosure, the four arginine residues corresponding to positions 250, 252, 256 and 258 of the receptor sequence are essential for IL-6 inhibition. In the following depiction of the sequences involved in the inhibition of IL-6, the underlined residues are those considered as essential:




Additional studies have shown that a peptide starting with leucine 255 of the IL-6R and extending beyond the known inhibitory peptide (249 through 258 of the IL-6R) towards the carboxy terminus, i.e., at the 3′ side, is also an effective inhibitor (Revel et al., 1995), indicating that not all four arginines are necessary for the inhibitory activity of this peptide sequence.

According to the present invention, libraries are designed to provide backbone cyclized peptidomimetics on these and other novel peptide sequences to optimall inhibit IL-6 bioactivity without disrupting normal ce functions, including those mediated via gp130 activat other growth factors or immunomodulators. Backbone c peptidomimetics are disclosed that achieve improved m stability and oral bioavailability without interferin the residues identified as essential for inhibitory function of these peptides.

Suitable targets for inhibition by peptidomimetics according to the present invention will include : 1) the interface between the IL-6 molecule and IL-6R; 2) the interface between IL-6 and gp130; 3) the interface between IL-6R and gp130. The peptide sequences to be modified are derived either from the IL-6R molecule, or from the IL-6 molecule itself.

Libraries of backbone cyclized peptidomimetics will be screened for inhibitory activity in known test systems for IL-6 action. The most active compounds will be isolated from the pooled libraries following individual assay. The IL-6 response can be conveniently measured in terms of growth

inhibition of myeloleukemic M1 cells. A well calibrated dose dependent inhibition of growth measured after 3 days of culture provides a quantitative assay of IL-6 action. Other cell types such as myelomas are growth stimulated and can also be used to quantitate the response to IL-6.

In order to optimize the activity, the length and cyclization state of the peptides is varied in order to mimic the conformation of the peptides as predicted by three dimensional computer models.

The initial libraries, are designed with the aim of identifying the best cyclization location and size within linear peptides derived from sequences 249-264 particularly 249-258 and 255-264 and peptides derived from other candidate contact positions of the IL-6R, the IL-6 and the gp130 molecules.

Example 27:

The first backbone-cyclic peptide library of residues 255-264 (YK-IL$\phi$1), contains 11 sub-libraries differ in the cyclization points as described in table X, leaving the Arg256, 258,261 unchanged and substituting the cyclization points amino acids with Gly-building units. The shortest distance between two bridgeheads is 5 amino acids.

Table X. Different cyclization points of YK-IL$\phi$1 library.

| | | | |
|---|---|---|---|
| 255-259 | 257-262 | 259-263 | 260-264 |
| 255-260 | 257-263 | 259-264 | |
| 255-262 | 257-264 | | |
| 255-263 | | | |
| 255-264 | | | |

Each sub-library contain 12 peptide with different building units at the cyclization points as indicated in Table XI.

Table XI. Peptides containing different bridge type and size.

| C-terminal unit / N-terminal unit | Gly-C1 | Gly-C2 | Gly-C3 | Gly-N2 | Gly-N3 |
|---|---|---|---|---|---|
| Gly-N2 | N2-C1 | N2-C2 | N2-C3 | | |
| Gly-N3 | N3-C1 | N3-C2 | N3-C3 | | |
| Gly-C1 | | | | C1-N2 | C1-N3 |
| Gly-C2 | | | | C2-N2 | C2-N3 |
| Gly-C3 | | | | C3-N2 | C3-N3 |

Example 28:

The second library is synthesized with the use of Arg-building units as cyclization points at positions 256, 258, 261 and use of the original sequence (Leu255, Ala259, etc.) modified amino acids as building units additional libraries are synthesized based on the biological information obtain from screening the initial libraries, using additional types of building units and substitution of other sequence amino acids.

Example 29:

The initial backbone-cyclic library of the 249-258 peptide (YK-IL(2), is composed of 7 sub-libraries, differ in the cyclization points (249-257, 249-255, 249-254, 257-253, 251-257, 251-255, 253-257), leaving the Arg250, 252,256,258 unchanged and substituting the cyclization points amino acids with 6 different Gly-building units yielding 12 peptides differ in their bridge, in each sub-library. The shortest distance between two bridgeheads is 5 amino acids.

In addition, backbone cyclic peptide libraries composed of shorter peptides of the above sequences (249-258 and 255-264), are synthesized with systematic deletions of amino acids that are not essential for activity.

Example 30: YS-IL-6 library

The library utilizes overlapping backbone cyclized decapeptides to scan the region of the IL-6 receptor that was found to have inhibitory activity. The overall sequence of this region divided into 12 overlapping decapeptides is illustrated in the following table where letters represent sublibraries and numbers represent original position in the IL-6 receptor.

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 269 | | | | | | | | | | | | Met |
| 268 | | | | | | | | | | | Trp | Trp |
| 267 | | | | | | | | | | Thr | Thr | Thr |
| 266 | | | | | | | | | Thr | Thr | Thr | Thr |
| 265 | | | | | | | | Phe | Phe | Phe | Phe | Phe |
| 264 | | | | | | | Thr | Thr | Thr | Thr | Thr | Thr |
| 263 | | | | | | Lys | Lys | Lys | Lys | Lys | Lys | Lys |
| 262 | | | | | Ser | Ser | Ser | Ser | Ser | Ser | Ser | Ser |
| 261 | | | | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Arg |
| 260 | | | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu |
| 259 | | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | |
| 258 | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Arg | | |
| 257 | Tyr | Tyr | Tyr | Tyr | Tyr | Tyr | Tyr | Tyr | Tyr | | | |
| 256 | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Arg | | | | |
| 255 | Leu | Leu | Leu | Leu | Leu | Leu | Leu | | | | | |
| 254 | Glu | Glu | Glu | Glu | Glu | Glu | | | | | | |
| 253 | Phe | Phe | Phe | Phe | Phe | | | | | | | |
| 252 | Arg | Arg | Arg | Arg | | | | | | | | |
| 251 | Leu | Leu | Leu | | | | | | | | | |
| 250 | Arg | Arg | | | | | | | | | | |
| 249 | Tyr | | | | | | | | | | | |

Each sublibrary represents one frame-shift of the IL-6 receptor sequence and contains ten analogs which differ in

their bridge as defined by the position of the building units (Gly-C2 and Gly-N3). The different bridges in each sublibrary are between the following positions (from peptide's N-termal): 2-6, 2-7, 2-8, 2-9, 3-7, 3-8, 3-9, 4-8, 4-9, 5-9.

For example, the format of sublibrary L, containing IL-6 receptor sequence 260-269 (with the original sequence in the left column, designated "L") is illustrated below:

| | L | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 269 | Met | Met | Met | Met | Met | Met | Met | Met | Met | Met | Met |
| 268 | Trp | GlyN3 | GlyN3 | GlyN3 | GlyN3 | Trp | Trp | Trp | Trp | Trp | Trp |
| 267 | Thr | Thr | Thr | Thr | Thr | GlyN3 | GlyN3 | GlyN3 | Thr | Thr | Thr |
| 266 | Thr | Thr | Thr | Thr | Thr | Thr | Thr | Thr | GlyN3 | GlyN3 | Thr |
| 265 | Phe | Phe | Phe | Phe | Phe | Phe | Phe | Phe | Phe | Phe | GlyN3 |
| 264 | Thr | GlyC2 | Thr | Thr | Thr | Thr | Thr | Thr | Thr | Thr | Thr |
| 263 | Lys | Lys | GlyC2 | Lys | Lys | GlyC2 | Lys | Lys | Lys | Lys | Lys |
| 262 | Ser | Ser | Ser | GlyC2 | Ser | Ser | GlyC2 | Ser | GlyC2 | Ser | Ser |
| 261 | Arg | Arg | Arg | Arg | GlyC2 | Arg | Arg | GlyC2 | Arg | GlyC2 | GlyC2 |
| 260 | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu | Glu |

The other eleven sublibraries were synthesized using the same format as shown above, by using the same building unit types at the corresponding positions within the particular amino acid subsequence from the original IL-6 receptor sequence. The library thus contain 12 sublibraries with 10 backbone cyclized analogs in each.

THE CLAIMS

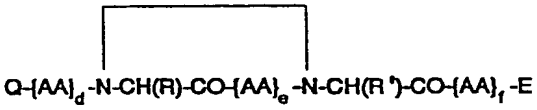
What is claimed is:

1. A library of chemical compounds comprising a plurality of backbone-cyclized peptide analogs, each compound comprising a peptide sequence having at least one building unit comprising an $N^{\alpha}$-derivative of an amino acid, wherein at least one backbone nitrogen in each said peptide sequence is linked to a side chain of at least one other amino acid in said peptide sequence or to at least one other backbone nitrogen in said peptide sequence by a bridging group comprising a disulfide, amide, thioether, thioester, imine, ether, or alkene bridge to form a backbone-cyclized peptide analog.

2. The library of claim 1 wherein at least one of said building units is located other than at the end of the peptide sequence.

3. The library of claim 1 wherein none of said building units is located at the end of the peptide sequence.

4. The library of claim 1 comprising a plurality of backbone-cyclized peptide analogs, wherein at least one pair of backbone nitrogens in each peptide sequence is linked together to form a peptide analog having the general formula (I):




Formula (I)

wherein: d, e, and f each independently designates 0 or an integer from 1 to 10; each {AA} designates an amino acid residue or the residue of a plurality of amino acids linked

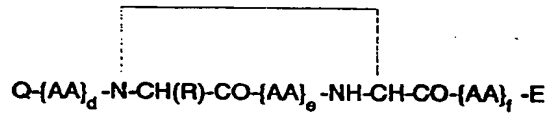
together through peptide bonding, wherein each {AA} may be the same or different; Q represents H or an acyl group; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the carboxy terminal group CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH or CHO; each of R and R' is independently hydrogen or an amino acid side-chain optionally bound with a specific protecting group; and the lines designate a bridging group of the formula:

(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein: M and W are independently selected from the group consisting of disulfide, amide, thioether, thioester, imine, ether, and alkene; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero-cycloalkylene and substituted cycloalkylene.

5. The library of claim 4 wherein -X-M-Y-W-Z- is: -(CH2)x-M-(CH2)y-W-(CH2)z- wherein M and W are as recited above; x and z each independently designates an integer of from 1 to 10, and y is zero or an integer of from 1 to 8, with the proviso that if y is zero, W is absent.

6. The library of claim 1 comprising a plurality of backbone-cyclized peptide analogs, wherein the backbone of each analog is cyclized to a side-chain of an amino acid to form a peptide analog of the general Formula (II):




Formula (II)

wherein: d, e, and f each independently designates 0 or an integer from 1 to 10; each {AA} designates an amino acid residue or the residue of a plurality of amino acids linked together through peptide bonding, wherein each {AA} may be the same or different; E represents a hydroxyl group, a

carboxyl protecting or an amino group, or CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH; R is an amino acid side chain optionally bound with a specific protecting group; and the line designates a bridging group of the formula:

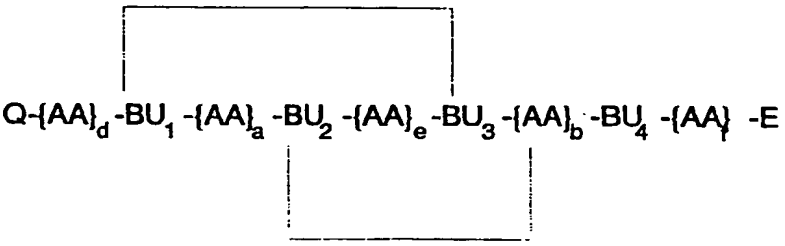
(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein M and W are independently selected from the group consisting of disulfide, amide, thioether, imine, ether, and alkene; X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene.

7. The library of claim 6 wherein -X-M-Y-W-Z- is: -(CH2)x-M-(CH2)y-W-(CH2)z- wherein M and W are as recited above; x and z each independently designates an integer of from 1 to 10, and y is zero or an integer of from 1 to 8, with the proviso that if y is zero, W is absent.

8. The library of claim 1 comprising a plurality of backbone-cyclized bicyclic peptide analogs, each of which comprises a plurality of building units comprising an $N^{\alpha}$-derivative of an amino acid.

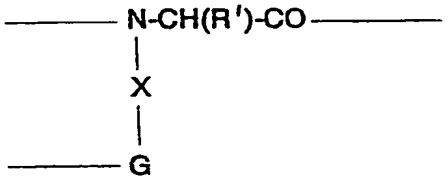
9. The library of claim 8 comprising a plurality of backbone-cyclized bicyclic peptide analogs each having the general formula (III):




Formula (III)

wherein: a and b each independently designates an integer from 1 to 8 or zero; d, e, and f each independently

designates an integer from 1 to 10 or zero; {AA} designates an amino acid residue -HN-CH(R)-CO- wherein R is an amino acid side chain, optionally bound with a specific protecting group, and the amino acid residues in each chain may be the same or different; Q represents H or an acyl group; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the carboxy terminal group CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH or CHO; BU represents an $N^{\alpha}$-$\omega$-functionalized derivative of amino acids of formula (IV):




Formula (IV)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is an amino acid side chain, optionally bound with a specific protecting group; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, and alkyl halides; which is incorporated into the peptide sequence and subsequently selectively cyclized via the functional group G with one of the side chains of the amino acids in said peptide sequence or with another $\omega$-functionalized amino acid derivative; and the lines designate a bridging group of the formula:

(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein: one of the lines may be absent; M and W are independently selected from the group consisting of disulfide, amide, thioether, thioester, imine, ether, and alkene; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene,

arylene, homo-cycloalkylene, or hetero-cycloalkylene and substituted cycloalkylene.

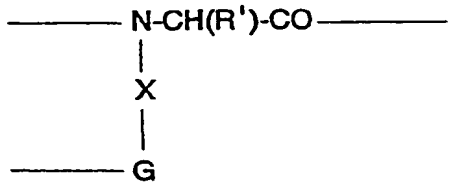
10. The library according to one of claims 4, 6, or 8 that has at least four members and wherein at least some of the analogs are bradykinin analogs, Substance P analogs, BPI analogs, somatostatin analogs, or interleukin-6 inhibitory peptide analogs.

11. The library according to one of claims 1, 4, 6, or 8 comprising two or more sublibraries, each containing a plurality of related peptide analogs.

12. A method for the preparation of a library of chemical compounds comprising a plurality of backbone-cyclized peptide analogs, each compound comprising a peptide sequence having at least one building unit comprising an $N^{\alpha}$-derivative of an amino acid, wherein at least one backbone nitrogen in each said peptide sequence is linked to a side chain of at least one other amino acid in said peptide sequence or to at least one other backbone nitrogen in said peptide sequence by a bridging group comprising a disulfide, amide, thioether, thioester, imine, ether, or alkene bridge to form a backbone-cyclized peptide analog, said method comprising the steps of:

providing peptide sequences having a plurality of building units containing amino acids and linked nitrogen atoms;

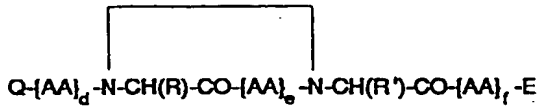
incorporating into each peptide sequence at least one $N^{\alpha}$-$\omega$-functionalized derivative of an amino acid of formula (IV):




Formula (IV)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R′ is an amino acid side chain, optionally bound with a specific protecting group; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, aldehydes, alcohols and alkyl halides, by selectively cyclizing a functional group G with another ω-functionalized amino acid derivative or with one of the side chains of the amino acids in said peptide sequence to form backbone-cyclized peptide analogs.

13. A method as in claim 12 for the preparation of a library of a plurality of backbone-cyclized peptide analogs of the general Formula (I):




Formula (I)

wherein: d, e, and f each independently designates an integer from 1 to 10; (AA) designates an amino acid residue wherein the amino acid residues in each chain may be the same or different; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the carboxy terminal group CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH; R and R′ each designates an amino acid side-chain

optionally bound with a specific protecting group; and the lines designate a bridging group of the formula:

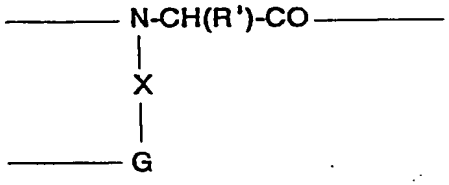
(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein: one line may be absent; M and W are independently selected from the group consisting of disulfide, amide, thioether, thioester, imine, ether, and alkene; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero-cycloalkylene and substituted cycloalkylene;

comprising the steps of:

providing peptide sequences having a plurality of building units containing amino acids and linked nitrogen atoms;

incorporating into each peptide sequence at least one $N^{\alpha}$-$\omega$-functionalized derivative of an amino acid of formula (IV):

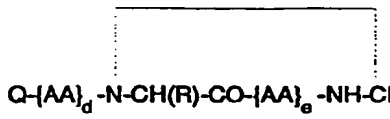



Formula (IV)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is an amino acid side chain, optionally bound with a specific protecting group; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, aldehydes, alcohols and alkyl halides, by selectively cyclizing the functional group G with another $\omega$-functionalized amino acid derivative to form backbone-cyclized peptide analogs.

14. The method of claim 13 wherein G is an amine, thiol, or carboxyl group.

15. A method as in claim 12 for the preparation of a library of a plurality of backbone-cyclized peptide analogs of the general formula (II):




Formula (II)

wherein d, e and f each independently designates an integer from 1 to 10; {AA} designates an amino acid residue wherein the amino acid residues in each chain may be the same or different; E represents a hydroxyl group, a carboxyl protecting or an amino group, or CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH; R is an amino acid side chain optionally bound with a specific protecting group; and the line designates a bridging group of the Formula:

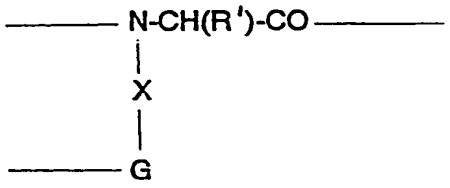
(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein M and W are independently selected from the group consisting of disulfide, amide, thioether, thioester, imine, ether, and alkene; X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero-cycloalkylene and substituted cycloalkylene;

comprising the steps of:

providing peptide sequences having a plurality of building units containing amino acids and linked nitrogen atoms;

incorporating into each peptide sequence at least one ω-functionalized derivative of an amino acid of formula (IV):




Formula (IV)

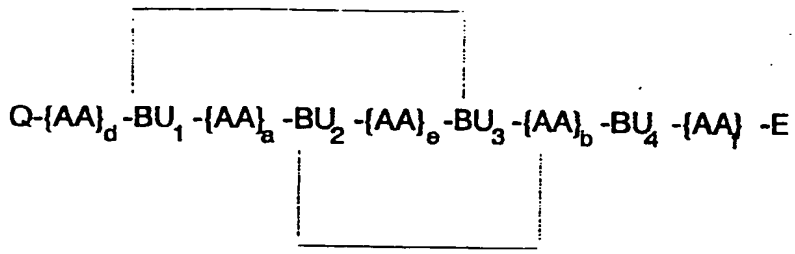
wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is the side chain of an amino acid; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters or alkyl halides, by selectively cyclizing the functional group G with one of the side chains of the amino acids in said peptide sequence to form backbone-cyclized peptide analogs.

16. The method of claim 15 wherein G is a carboxyl group or a thiol group.

17. The method of one of claims 13 or 15, wherein R is CH3-, (CH3)2CH-, (CH3)2CHCH2-, CH3CH2CH(CH3)-, CH3S(CH2)2-, HOCH2-, CH3CH(OH)-, HSCH2-, NH2C(=O)CH2-, NH2C(=O)(CH2)2-, NH2(CH2)3-, HOC(=O)CH2-, HOC(=O)(CH2)2-, NH2(CH2)4-, C(NH2)2 NH(CH2)3-, HO-phenyl-CH2-, benzyl, methylindole, or methylimidazole.

18. A method as in claim 12, wherein the library comprises a plurality of backbone-cyclized bicyclic peptide analogs, each of which comprises a plurality of building units comprising an $N^{\alpha}$-derivative of an amino acid.

19. A method as in claim 18 for the preparation of a library of a plurality of backbone-cyclized bicyclic peptide analogs of the general Formula (III):




Formula (III)

wherein: d, e, and f each independently designates an integer from 1 to 10; (AA) designates an amino acid residue wherein the amino acid residues in each chain may be the same or different; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the carboxy terminal group CO-E, wherein the CO is part of {AA}, can be reduced to $CH_2$-OH; R and R′ each designates an amino acid side-chain optionally bound with a specific protecting group; and the lines designate a bridging group of the Formula:

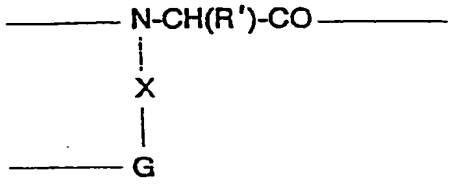
(i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-

wherein: one line may be absent; M and W are independently selected from the group consisting of disulfide, amide, thioether, thioester, imine, ether, and alkene; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero- cycloalkylene and substituted cycloalkylene;

comprising the steps of:

providing peptide sequences having a plurality of amino acids;

incorporating into each peptide sequence at least one $N^{\alpha}$-ω-functionalized derivative of an amino acid of Formula (IV):



Formula (IV)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is an amino acid side chain, optionally bound with a specific protecting group; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, aldehydes, alcohols and alkyl halides, by selectively cyclizing a functional group G with another ω-functionalized amino acid derivative or with one of the side chains of the amino acids in said peptide sequence to form backbone-cyclized peptide analogs and further cyclizing a second functional group G with yet another ω-functionalized amino acid derivative or with one of the side chains of the amino acids in said peptide sequence to form backbone-cyclized bicyclic peptide analogs.

20. The method of any one of claims 13, 15, or 18 wherein said peptide sequences are provided covalently coupled to an insoluble polymeric support.

21. A method of screening for active peptide analogs comprising:

(a) generating a library of chemical compounds according to claim 12 wherein members of the library vary in at least one of the following: (i) positions in the linear peptide sequence of residues that are cyclized, (ii) length of the bridge between the residues, (iii) direction of the

bridge between the residues, and (iv) bond type of the bridge between the residues;

(b) testing the members of the library for biological activity; and

(c) identifying the active members of the library.

22. The method of claim 21 wherein the members of the library are tested for somatostatin activity.

23. The method of claim 22 wherein the somatostatin activity is measured by determining the ability of the members to bind to a somatostatin receptor.

24. The method of claim 22 wherein the positions in the linear peptide sequence of residues that are cyclized correspond to positions 6 and 11 of natural somatostatin.

25. A method of screening compounds which comprises forming a library of at least four backbone-cyclized peptide analogs as described in one of claims 1, 4, 6, or 8, and screening the analogs for activity as bradykinin agonists or antagonists, Substance P analogs, BPI analogs, somatostatin agonists or antagonists, or interleukin-6 inhibitory peptide analogs.